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(54) **Title:** METHOD OF PROVIDING PROPHYLAXIS FOR TUBERCULOSIS IN HIV POSITIVE INDIVIDUALS

(57) **Abstract:** Present invention relates to the method providing prophylaxis for tuberculosis in HIV positive individuals. According to present invention, vaccine made from 'Mycobacterium w' (Mw) is found to be useful in providing prophylaxis against tuberculosis in HIV positive individuals.

S.No.	Age	Mantoux Test	
		Baseline	Day 90
1.	28	0 mm	16 mm
2.	24	0 mm	14 mm
3.	33	0 mm	6 mm
4.	20	0 mm	15 mm
5.	25	0 mm	15 mm
6.	30	0 mm	14 mm
7.	35	0 mm	6 mm
8.	40	0 mm	7 mm
9.	27	0 mm	17 mm
10.	31	0 mm	12 mm

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THE METHOD PROVIDING PROPHYLAXIS FOR TUBERCULOSIS IN HIV POSITIVE INDIVIDUALS

Tuberculosis is a major communicable disease worldwide. It is caused by mycobacterium tuberculosis. It is a major cause of morbidity and mortality worldwide which includes developing countries as well as developed countries. This is happening inspite of availability of effective chemotherapy.

The problem of tuberculosis has gained more attention recently due to spreading epidemic of tuberculosis worldwide. The immunity in HIV is compromised and it makes the individual more vulnerable to various infectious disease particularly tuberculosis. The decrease in immunity is more pronounced for cell mediated immunity than humoral immunity. The incidence of tuberculosis is much more in HIV positive individuals compared to normal subjects. It varies from 32% in Brazil to 64% in India in HIV +ve individuals. The increased risk of tuberculosis can also be judged by the fact that in normal individuals risk of tuberculosis is 5% in 5 years compared to 8% in in first year in HIV positive. Similarly if life time risk of developing tuberculosis is one in normal individuals than it is 113 in HIV positive individuals.

Thus there is a greater need to provide prophylaxis against tuberculosis in HIV positive individuals.

The immunity against tuberculosis is judged by a test called tuberculin test. It is performed by injecting antigens [purified protein derivative (PPD)] of mycobacterium tuberculosis. In persons having immunity against tuberculosis there develops reaction at site of injection, which is read at 48 to 72 hours after infection. The reaction which develops at injection site consists of a raised, red, and hard (indurate) area in the skin. This is indicative of presence of cell mediated immunity against tuberculosis.,

The immunity as detected by this method is found in individuals who are given BCG vaccination or exposed to tuberculosis organisms.

The only known vaccine in use for providing prophylaxis against tuberculosis is BCG. The BCG contains live microorganisms and so it can not be given to immunocompromised individuals like HIV positive individuals. The current recommendations are to provide prophylaxis to HIV positive individuals by chemotherapeutic agents like Isoniazid, Rifampicin etc. There is no accepted method for providing immunity against tuberculosis. Thus there is unmet requirement for providing immunity against tuberculosis in HIV positive individuals.

US patents 54724144, 5985287, 6160093, 6001361 describes use of mycobacterium vaccae or its various components effective for the purpose of providing immunity against tuberculosis in animals.

US patent 6210684 and WO 9406466 describes use of mycobacterium vaccae for treatment or prophylaxis of AIDS.

However when used in human who are HIV positive mycobacterium vaccae fails to provide immunity against tuberculosis even after 3 to 5 doses. (Johnson D et al. Vaccine 1999, 17(20-21): 2583-7: Marsha BJ et. al. Am J Med Sci 1997, 313(6):377-83,: Waddell RD, Clin Infect Dis 2000, 30 Suppl 3:s309-15)

Thus the need to provide immunity against tuberculosis in HIV positive individuals is not met.

The failure to elicit immune response with mycobacterium vaccae may be due to inability of depleting CD4 cells to function in a manner to improve cell mediated immunity against tuberculosis which is judged by tuberculin conversion.

Surprisingly according to present invention it is observed that it is possible to provide a pharmaceutical composition for immunity against tuberculosis in HIV positive individuals. The process of preparing pharmaceutical composition for this purpose involves use of mycobacterium w.

Mycobacterium w is found to be useful in management of leprosy. It converts lepromin negative individuals to lepromin positive status. It also reduces the duration of therapy required for cure of multibacillary leprosy.

The pharmaceutical composition made as per present invention is found to be effective in providing immunity against tuberculosis in HIV positive individuals as judged by tuberculin test.

Summary of the invention

According to present invention, vaccine made from 'Mycobacterium w' (M_w) is found to be useful in providing prophylaxis against tuberculosis in HIV positive individuals. It is observed that administration of mycobacterium w containing vaccine is capable of converting tuberculin negative and hiv positive individuals into tuberculin positive status. These effects have been found in patients suffering from tuberculosis also. These effects are also seen in patients who are suffering from HIV infection with or without AIDS and with or without associated tuberculosis.

Mycobacterium w used in the present invention is a non-pathogenic, cultivable, atypical mycobacterium, with biochemical properties and fast growth characteristics resembling those belonging to Runyons group IV class of Mycobacteria in its metabolic and growth properties but is not identical to those strains currently listed in this group. It is therefore thought that (M_w) is an entirely new strain.

The species identity of Mw has been defined by polymerase chain reaction DNA sequence determination and differentiated from thirty other species of mycobacteria. It however differs from those presently listed in this group in on respect or the other. By base sequence analysis of a polymorphic region of pattern analysis, it has been established that M_w is a unique species distinct from many other known mycobacterial species examined which are: M. avium, M. intracellulare, M. scrofulaceum, M. kansasii, M. gastri, M. gordonae, M. shimoidei, M. malmoense, M. haemophilum, M. terrae, M. nonchromogenicum, M. triviale, M. marinum, M. flavescens, M. simian, M.

szulgai, M. xenopi, M. asciaticum, M. aurum, M. smegmatis, M. vaccae, M. fortuitum subsp fortuitum, M. fortuitum subsp. Peregrinum, M. chelonae subsp. Chelonae, M. chelonae subsp. Abscessus, M. genavense, M. tuberculosis, M. tuberculosis $H_{37}R_{v,}$ M. paratuberculosis.

The object of the present invention is to provide a vaccine containing 'Mycobacterium w' (Mw) with or without constituents obtained from Mw for the prophylaxis against tuberculosis, to a subject exposed to HIV infection or is HIV positive with or without overt symptoms of AIDS.

Yet another object of the invention is to provide a vaccine to convert tuberculin negative individuals who are HIV positive to tuberculin positive status.

Yet another object of the invention is to provide vaccine derived from Mycobacterium w to improve tuberculin status of HIV +ve subjects.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention the composition of immunomodulator the method of preparation, HPLC characteristic its safety and tolerability, methods of use and outcome of treatments are described in following examples. The following are illustrative examples of the present invention and scope of the present invention should not be limited by them.

Example 1. The pharmaceutical compositions:

A. Each dose of 0.1 ml of therapeutic agent contains:

Mycobacterium w., (heat killed)	0.50×10^9
Sodium Chloride I. P.	0.90% w/v
Tween 80	0.1% w/v
Thiomerosal I. P.	0.01% w/v
(As a Preservative)	
Water for injection I. P.	q. s. to 0.1 ml

B. Each dose of 0.1 ml of therapeutic agent contains:

Mycobacterium w., (heat killed)	0.50×10^9
Sodium Chloride I. P.	0.90% w/v
Triton x 100	0.1% w/v
Thiomerosal I. P.	0.01% w/v
(As a Preservative)	
Water for injection I. P.	q. s. to 0.1 ml

C. Each dose of 0.1 ml of therapeutic agent contains:

Mycobacterium w., (heat killed)	0.50×10^9
Sodium Chloride I. P.	0.90% w/v
Thiomerosal I. P.	0.01% w/v
(As a Preservative)	
Water for injection I. P.	q. s. to 0.1 ml

D. Each dose of 0.1 ml of therapeutic agent contains

Extract of Mycobacterium w after sonication from $1x10^{10}$ Mycobacterium w

Sodium Chloride I. P.	0.90% w/v
Thiomerosal I. P.	0.01% w/v
(As a Preservative)	
Water for injection I. P.	q. s. to 0.1 ml

E. Each dose of 0.1 ml of therapeutic agent contains

Methanol Extract of $1x10^{10}$ Mycobacterium w

Sodium Chloride I. P.	0.90% w/v
Thiomerosal I. P.	0.01% w/v
(As a Preservative)	
Water for injection I. P.	q. s. to 0.1 ml

F. Each dose of 0.1 ml of therapeutic agent contains

Chloroform Extract of $1x10^{10}$ Mycobacterium w

Sodium Chloride I. P. ... . 0.90% w/v

Thiomerosal I. P. ... . 0.01% w/v

(As a Preservative)

Water for injection I. P. q. s. to 0.1 ml

G. Each dose of 0.1 ml of therapeutic agent contains

Acetone Extract of $1x10^{10}$ Mycobacterium w

Sodium Chloride I. P. ... . 0.90% w/v

Thiomerosal I. P. ... . 0.01% w/v

(As a Preservative)

Water for injection I. P. q. s. to 0.1 ml

H. Each dose of 0.1 ml of therapeutic agent contains

Ethanol Extract of $1x10^{10}$ Mycobacterium w

Sodium Chloride I. P. ... . 0.90% w/v

Thiomerosal I. P. ... . 0.01% w/v

(As a Preservative)

Water for injection I. P. q. s. to 0.1 ml

I. Each dose of 0.1 ml of therapeutic agent contains

Liticase Extract of $1x10^{10}$Mycobacterium w

Sodium Chloride I. P. ... . 0.90% w/v

Thiomerosal I. P. ... . 0.01% w/v

(As a Preservative)

Water for injection I. P. q. s. to 0.1 ml

J. Each dose of 0.1 ml of therapeutic agent contains

Mycobacterium w (heat killed) $0.5x10^{7}$

Extract of mycobacterium w obtained $1x10^{3}$ Mycobacterium w by disruption, solvent extraction or enzymatic extraction.

Sodium Chloride I. P.	... .	0.90% w/v
Thiomerosal I. P.	... .	0.01% w/v
(As a Preservative)		
Water for injection I. P.		q. s. to 0.1 ml

Example 2. The Process of preparing a pharmaceutical composition

A. Culturing of Mycobacterium w.

i) Preparation of culture medium.

Mycobacterium w is cultured on solid medium like L J medium or liquid medium like middle brook medium or sauton's liquid medium. For better yield middle brook medium is enriched. It can be preferably enriched by addition of glucose, bactotryptone, and BSA. They are used in ratio of 20:30:2 preferably.

The enrichment medium is added to middle brook medium. It is done preferably in ratio of 15:1 to 25:1 more preperably in ratio of 20:1.

ii) Bioreactor operation

a). Preparation of vessel:

The inner contact parts of the vessel (Joints, mechanical seals, o-ring/gasket grooves, etc.) should be properly cleaned to avoid any contamination. Fill up the vessel with 0.1 N NaOH and leave as such for 24 H to remove pyrogenic materials and other contaminants. The vessel is then cleaned first with acidified water, then wit ordinary water. Finally, the vessel is rinsed with distilled water (3 times) before preparing medium.

b) Sterilization of bioreactor

The bioreactor containing 9L distilled water is sterilized with live steam(indirect). Similarly the bioreactor is sterilized

once more with Middlebrook medium. The other addition bottles, inlet/outlet air filters etc. are autoclaved (twice) at 121^{0}C for 15 minutes. Before use, these are dried at 50^0 C oven.

c) Environmental parameter

i. Temprature: 37± 0.5^0 C

ii. pH : 6.7 to 6.8 initially.

B. Harvesting and concentrating

It is typically done at the end of 6th day after culturing under aseptic condition. The concentration of cells (palletisation) is done by centrifugation.

C. Washing of cells

The pallet so obtained is washed minimum three times with normal saline. It can be washed with any other fluid which is preferably isotonic.

D. Adding pharmaceutically acceptable carrier.

Pyrogen free normal saline is added to pallet. Any other pyrogen free isotonic fluid can be used as a pharmaceutical carrier. The carrier is added in amount so as get to desired concentration of active in final form.

E. Adding preservative

To keep the product free from other contaminating bacteria for its self life preservative is added. Preferred preservative is thiomesol which is used in final concentration of 0.01 % w/v.

F. Terminal Sterilization

Terminal sterilization can done by various physical methods like application of heat or ionizing radiation or sterile filtration.

Heat can be in the form of dry heat or moist heat. It can also be in the form of boiling or pasturisation.

Ionizing radiation can be ultraviolet or gamma rays or mircrowave or any other form of ionizing radiation.

It is preferable to autoclave the final product.

This can be done before after filling in a final packaging.

G. Quality Control

i. The material is evaluated for purity, sterility.

ii. The organisms are checked for acid fastness after gram staining.

iii. Inactivation test : This is done by culturing the product on L J medium to find out any living organism.

iv. Pathogenicity and/or contamination with pathogen.

The cultured organisms are infected to Balb/c mice.

None of the mice should die and all should remain healthy and gain weight. There should not be any macroscopic or microscopic lesions seen in liver, lung spleen or any other organs when animals are killed upto 8 weeks following treatment.

v. Biochemical Test:

The organism is subjected to following biochemical tests:

a) Urease

b) Tween 80 hydrolysis

c) Niacin test

d) Nitrate reduction test

The organism gives negative results in urease, tween 80 hydrolysis and niacin test. It is positive by nitrate reduction test.

H. Preparation of constituents of Mycobacterium w.

The constituents of Mycobacterium w can be prepared for the purpose of invention by:

I. Cell disruption

II. Solvent extration

III. Enzymatic extraction.

The cell disruption can be done by way of sonication or use of high pressure fractionometer or by application of osmotic pressure ingredient.

The solvent extraction can be done by any organic solvent like chloroform, ethanol, methanol, acetone, phenol, isopropyl alcohol, acetic acid, urea, hexane etc.

The enzymatic extraction can be done by enzymes which can digest cell wall/membranes. They are typically proteolytic in nature. Enzyme liticase and pronase are the preferred enzymes. For the purpose of invention cell constituents of Mycobacterium w can be

used alone in place of mycobacterium w organisms or it can be added to the product containing mycobacterium w.

Addition of cell constituents results in improved efficacy of the product.

Example 3. Characteristics of constituents of Mycobacterium w by HPLC analysis.

The constituents of mycobacterium w. used for the purpose of invention when subjected to HPLC analysis gives a single peak at 11 minutes. No other significant peaks are found beyond. The peak is homogenous and devoid of any notch suggesting homogeneity of material obtained

HPLC analysis was done using a waters system high performance liquid chromatography apparatus

Column:	Novapak c1860A, 4μm, 3.9 x 150mm.
The guard column:	Novapak c 18
Column Temperature:	30^0 c
Flow rate:	2.5 ml/min
Injection volume:	25μL.

Mobile phase:

Solvent A: HPLC grade methanol.

Solvent B: HPLC grade methylene chloride

Binary gradient:

The HPLC gradient initially comprised 98%(v/v) methanol (solvent B).

The gradient was increased linearly to 80%.

A and 20% B at one minute; 35% A and 65% B at 10 minutes, held for 5 seconds and then decreased over 10 seconds back to 98% A and 2% B.

Example 4. Immunity against tuberculosis in HIV sero positive individuals

Ten HIV positives (subjects) were enrolled in this study. All of them were tuberculin negative with a tuberculin reading of '0' m.m. and that was the reason of including them in study. All were administered intradermal mycobacterium w.. In all subjects, tuberculin test to determine tuberculin like delayed-type hypersensitivity reaction was repeated after ninety days.

Results of the study are shown in Fig1. In all 10 subjects repeat tuberculin test performed after 90 days revealed a reading of more than 5 m.m. In 8 of 10 subjects it was more than 10 m.m. Maximum reading seen was 17 m.m. and minimum was 6 m. m. The mean reading was 12.6 m.m.

In HIV positive individuals cut-off point for considering an individual tuberculin positive is 5 m.m. thus all the subjects got converted from tuberculin negative status to tuberculin positive status. Thus in all subjects immunity against mycobacterium tuberculosis as determined by tuberculin conversion from negative to positive was obtained after single intradermal injection.

The tuberculin negative status as seen in this study before enrollment is seen in spite of patients having active tuberculosis.
In HIV positive individuals immunity decreases with decrease in CD4 count. This decreased cell mediated immunity results in change in tuberculin status also. Initialyy tuberculin positive subjects become tuberculin negative with decrease in immunity.
In immunocompetent individuals tuberculosis can be diagnosed by positive tuberculin test in an individual who neither given BCG nor exposed to tuberculosis. Thus tuberculin negativity '0' m.m. reading inspite of active tuberculosis suggests difficult situation for tuberculin conversion.
The present invention provides tuberculin conversion and immunity against tuberculosis in highly vulnerable group and provides prophylaxis, a much desired effect.

We Claim:

1. A method of providing immunity against tuberculosis in HIV positive individuals comprises administration of a formulation which is prepared using Mycobacterium w or a pharmaceutical composition obtained from Mycobacterium w alone or in combination and also with or without adjuvants to a subject who is HIV positive.

2. The method as claimed in claim 1 for providing immunity against tuberculosis in HIV positive individuals is effective converting tuberculin negative individuals to tuberculin positive individuals.
3. The method as claimed in claim 1 for providing immunity against tuberculosis in HIV positive individuals is effective in improving tuberculin status of treated individuals.

4. The product as claimed in claim 1 contain mycobacterium w is killed mycobacterium w.
5. The Mycobacterium w as claimed in claim 1 and 2 is killed by physical method like, heat radiation most preferably by heat in form of autoclaving.
6. The product as claimed in claim 1 is obtained from mycobacterium w by sonication.
7. The product as claimed in claim 1 is obtained from mycobacterium w by extraction.
8. The product as claimed in claim 1 and 5 is obtained from mycobacterium w is extracted by organic solvents.
9. The product as claimed in claim 1, 5 and 6 is extracted using solvent selected from chloroform, ethanol, methanol, acetone, phenol, isopropyl alcohol, acetic acid, urea, Hexane and like.
10. The adjuvants as claimed in claim 1 is selected from mineral oil, mineral oil and surfactant, Ribi adjuvant, Titer-max, syntax adjuvant formulation, aluminium salt adjuvant, nitrocellulose adsorbed antigen, immune stimulating complexes, Gebru adjuvant, super carrier, elvax 40w, L –tyrosine, monatanide (manide –oleate compound), Adju prime, Squalene, Sodium phthalyl lipopoly saccharide, calcium phosphate, saponin, melanoma antigen, muramyl dipeptide(MDP) and like.
11. The formulation as claimed in claim 1 contains surfactant.
12. The surfactant as claimed in claim 9 can be a Tween 80.
13. The amount of surfactant as claimed in claim 9 and 10 is upto 0.4% preferably 0.1%.
14. The formulation as claimed in claim1 containing mycobacterium w or obtained from mycobacterium w or combination of both with or without adjuvants helps in amelioration of symptoms of cancer.
15. The formulation as claimed in claim1 containing mycobacterium w or obtained from mycobacterium w or combination of both with or without adjuvants are capable of causing regression or even complete control of cancer.
16. The Mycobacterium w as claimed in claim 1,2,3,4,5,6 is a non-pathogenic, fast growing cultivable, atypical mycobacterium, with biochemical properties and growth characteristics resembling those

belonging to Runyons group IV class of Mycobacteria in its metabolic and growth properties but is not identical to those strains currently listed in this group.

17. Mycobacterium w as claimed in claim 1 is urease negative, does not hydrolyse tween 80, does not produce niacin, provides strong positive response to nitrate reduction test.
18. The cancerous tissue as claimed in claim 17 can be a primary or a secondary(metastatic) lesion.
19. The method as claimed in claim 1 is effective in reducing side effects of other cancer therapies like radiotherapy, chemotherapy.
20. The administration of formulation as claimed in claim 1 is by parental route.
21. The administration as claimed in claim 1 and 17 is by intramuscular subcutaneous, intradermal route and like but preferably by intradermal route.
22. The amount of mycobacterium w administered at a time to a subject as claimed in claim 1 is equal to or more than $1x\ 10^5$ mycobacterium w.
23. The amount of mycobacterium w administered at a time to a subject as claimed in claim 1 is equal to or more than 10^7 mycobacterium w.
24. The amount of mycobacterium w administered at a time to a subject as claimed in claim 1 is most preferably $1x\ 10^8$ to $1x\ 10^{10}$ mycobacterium w.
25. The process of manufacturing a pharmaceutical composition useful for management of cancer comprises of incorporating cells of mycobacterium w alongwith pharmaceutically acceptable carrier and optionally a preservative in a single formulation wherein cells of mycobacterium w are not alive.
26. The pharmaceutically acceptable carrier as claimed in clain 1 is added in a way so as to have more than or equal to $1x\ 10^5$ mycobacterium w in a unitary dosage, more preferably equal to or more than $1x10^7$ mycobacterium w in unitary dosage most preferably between $1x10^8$ to $1x10^9$ cells of mycobacterium w in a unitary dosage form.
27. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising the steps of incorporating disrupted cells of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.
28. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising the steps of incorporating solvent extraction of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative.
29. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising of incorporating enzymatic extraction of mycobacterium w along with pharmaceutically acceptable carrier and optionally a preservative
30. The process of manufacturing a pharmaceutical composition useful for management of cancer comprising admixing product of claim 1 with product of claim 31 and/or claim 32 and/ or claim 33.
31. The process of manufacturing a pharmaceutical composition useful for management of cancer comprise of adding adjuvant to product of claim 1, claim 4, claim 6, claim 8 or claim 10.

32. The adjuvant as claimed in claim 17 is selected from mineral oil, mineral oil and surfactant, Ribi adjuvant, Titer-max, syntax adjuvant formulation, aluminium salt adjuvant, nitrocellulose adsorbed antigen, immune stimulating complexes, Gebru adjuvant, super carrier, elvax 40w, L –tyrosine, monatanide (manide –oleate compound), Adju prime, Squalene, Sodium phthalyl lipopoly saccharide, calcium phosphate, saponin, melanoma antigen, muramyl dipeptide(MDP) and like.

Table 1

S.No.	Age	Mantoux Test	
		Baseline	Day 90
1.	28	0 mm	16 mm
2.	24	0 mm	14 mm
3.	33	0 mm	6 mm
4.	20	0 mm	15 mm
5.	25	0 mm	15 mm
6.	30	0 mm	14 mm
7.	35	0 mm	6 mm
8.	40	0 mm	7 mm
9.	27	0 mm	17 mm
10.	31	0 mm	12 mm

Fig. 1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/00207-0

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: A61K 39/04, 39/39, A61P 31/06, 35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: A61K 39/04, 39/39, A61P 31/06, 35/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, CAS, Medline

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94/06466 A1 (UNIVERSITY COLLEGE LONDON) 31 March 1994 (31.03.94) *claims.*	1-17,19-24,26,31
A	LUO, Y et al. Immunotherapeutic effect of Mycobacterium vaccae on multi-drug resistant pulmonary tuberculosis. Zhonghua jie he he hu xi za zhi = Zhonghua jiehe he huxi zazhi = Chinese journal of tuberculosis and respiratory diseases, February 2000, Vol. 23, No. 2, pages 85-88, Medline-abstract [online], [retrieved on 7 May 2003 (07.05.03)]. Retrieved from: EPOQUE Medline Database, AN: NLM11778496 *abstract.*	1-17,19-24,26,31

☒ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
„A" document defining the general state of the art which is not considered to be of particular relevance
„E" earlier application or patent but published on or after the international filing date
„L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
„O" document referring to an oral disclosure, use, exhibition or other means
„P" document published prior to the international filing date but later than the priority date claimed
„T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
„X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
„Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 May 2003 (07.05.2003)	2 July 2003 (02.07.2003)
Name and mailing adress of the ISA/AT Austrian Patent Office Dresdner Straße 87, A-1200 Vienna Facsimile No. 1/53424/535	Authorized officer MOSSER R. Telephone No. 1/53424/437

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/00207-0

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GULERIA, I. et al. In vivo depletion of CD4 and CD8 T lymphocytes impairs Mycobacterium w vaccine-induced protection against M. tuberculosis in mice. Medical microbiology and immunology, July 1993, Vol. 182, No. 3, pages 129-135, Medline-abstract [online], [retrieved on 7 May 2003 (07.05.03)]. Retrieved from: EPOQUE Medline Database, AN: NLM7901743 *abstract.* ----	1-17,19-24,26,31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/00207-0

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 18,30,32
because they relate to subject matter not required to be searched by this Authority, namely:
see extra sheet

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1.) Claims 1-17, 19-24, 26 and 31 concern methods and products derived from a mycobacterium for providing immunity against tuberculosis in HIV positive individuals.

2.) Claims 25 and 27-29 concern the treatment of cancer with mycobacteria. These claims are not dependent from claim 1 and do not concern tuberculosis and HIV positive patients.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Extra sheet

Box I, 1.:

The subject matters of claims 18, 30 and 32 are not clear:
Claim 18 which is dependent from claim 17 concerns cancerous tissue. But claim 17 relates to mycobacteria.
Claim 30 concerns the mixing of "product of claim 1" with further products. However, it is not clear which are the further products (adjuvants etc.); and there does not exist a claim 33.
Claim 32 concerns adjuvants and further compounds such as antigens and is also dependent from claim 17. The subject matter of this claim is not clear as well.

Remark: Although claims 1-3 and 19-24 concern the treatment of the human body by therapy (see PCT Rule 39.1(iv) the search was carried out and based on the alleged effects.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB 03/00207-0

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